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In Search of an Effective *in vivo* Reactivator for Organophosphorus Nerve Agent-Inhibited Acetylcholinesterase in the Central Nervous System[1]

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[1] The views expressed in this article are those of the authors and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

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14. ABSTRACT
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Abstract

Organophosphorus nerve agents irreversibly inhibit the enzyme acetylcholinesterase (AChE), leading to excessive cholinergic neurotransmission and causing toxic lethal consequences. Current nerve agent therapies in the U.S. include 2-pralidoxime to reactivate inhibited AChE. Due to its quaternary structure, this oxime does not readily cross the blood brain barrier (BBB) to reactivate brain AChE and to mitigate CNS toxicity. We have shown earlier that the tertiary oxime monoisonitrosoacetone (MINA) crossed BBB, provided some degree of CNS AChE reactivation, enhanced survival, and mitigated the seizure activity following nerve agent exposure. In this study, the *in vivo* reactivating capabilities of several tertiary oximes, diacylmonoxime (DAM), N,N-diethyl-3-(2-(hydroxyimino)acetoxy)propan-1-aminium chloride (DHAP), RS194B, JK-3-38, SWRI48A, SWRI53A, pro-2-PAM, and diethyxime, were compared to each other and to MINA, following subcutaneous exposure of guinea pigs to 1.0 x LD_{50} dose of sarin (GB), cyclosarin (GF) or VX. Four to eight doses (ranging from 5.6 to 240.0 mg/kg, i.m.) of each oxime were treated 15 min after the nerve agent exposure. Animals were euthanized 45 min after oxime treatment; blood and target tissues (brain regions, diaphragm, heart, skeletal muscle) were collected. AChE activity was measured using the Ellman assay. In GB exposure, pro-2-PAM and JK-3-38 enhanced the toxicity at doses above 25 and 35.5 mg/kg, respectively. In peripheral tissues pro-2-PAM provided the greatest AChE reactivation (21-68%), while SWRI53A showed reactivation (4%) only at the highest dose (180 mg/kg), with the rank order of pro-2-PAM > RS194B = JK-3-38 > DHAP > MINA > diethyxime > DAM = SWRI48A = SWRI53A. In the CNS tissues, MINA displayed the highest capacity to reactivate AChE (15-31%), while DAM and JK-3-38 provided no reactivation even at the highest dose tested; the rank order was MINA > pro-2-PAM > DHAP > SWRI48A = SWRI53A > RS194B = diethyxime > JK-3-38 = DAM. In GF exposure, only MINA and SWRI48A showed brain AChE reactivation (8-17%), while only MINA and pro-2-PAM reactivated VX-inhibited brain AChE (8-23%). Thus, the findings suggest that, like the quaternary oximes, tertiary oximes exhibit different reactivation capacities that depend on the nerve agent inhibiting AChE. So far, MINA is the only tertiary oxime capable of reactivating brain AChE inhibited by these three nerve agents.

Keywords: acetylcholinesterase; brain; cholinesterase inhibition; cholinesterase reactivation; cyclosarin; diacetylmonoxime; diethyxime; dihydro-2-PAM; guinea pig; methoxime; monoisonitrosoacetone; nerve agents; organophosphorus compounds; oximes; pralidoxime; sarin; VX

INTRODUCTION

Organophosphorus (OP) nerve agents, such as sarin (GB), cyclosarin (GF), and VX, are potent inhibitors of the enzyme cholinesterase (ChE). Their toxic effects are due to hyperactivity of the cholinergic system as a result of ChE inhibition, especially acetylcholinesterase (AChE), and the subsequent increase in the level of the neurotransmitter acetylcholine (ACh) in the central nervous system (CNS) and periphery [Taylor, 2001]. In the event of nerve agent poisoning, immediate medical therapy consists of an anticholinergic such as atropine sulfate to antagonize the effects of ACh at muscarinic receptors, an oxime such as 2-PAM (pralidoxime), obidoxime (Toxogonin®), or HI-6 to reactivate any unaged, inhibited enzyme, and an anticonvulsant such as diazepam or midazolam to control convulsions and brain seizures [Wilson and Ginsburg, 1955; Moore et al., 1995; Taylor, 2001; Aas, 2003]. The brain is a major target for the toxic effects of nerve agents. Inhibition of AChE in the brain results in seizures and neuropathology and contributes to the incapacitating behavioral and lethal effects of these agents [McDonough and Shih, 1997; Shih et al., 2003, 2007]. The currently used oximes (i.e., 2-PAM, obidoxime, or HI-6) can reactivate ChEs in peripheral tissues but are quaternary nitrogen compounds with limited ability to cross the blood brain barrier (BBB). Thus, the inability to enter the CNS and reactivate nerve agent-inhibited brain AChE is a major limitation of current oxime therapy. Protecting and/or restoring AChE activity in the brain is a major goal in the treatment of nerve agent intoxication. Our long-term goal is to develop a safe, effective, and broad-spectrum *in vivo* reactivator for nerve agent-inhibited AChE in the CNS. Here we report the status of nine tertiary oximes that were capable of entering the CNS (see Figure 1 for chemical structures).

The study of CNS acting AChE reactivators for OP agent exposure has been formerly investigated. Monoisonitrosoacetone (MINA) and diacetylmonoxime (DAM; 2,3-butanedione monoxime) are two tertiary oximes that were investigated in the 1950s. Both are highly lipid soluble and readily penetrate the BBB [Cohen and Wiersinga, 1960], and are capable of reactivating AChE within the CNS [Rutland, 1958; Cohen and Wiersinga, 1960]. Indeed, when used alone or in combination with atropine sulfate, MINA and DAM were shown to raise the LD_{50} doses of GB in several animal species [Askew, 1956a; 1957; Dultz et al., 1957; Rutland, 1958; Myers, 1959; Wills, 1959]. Unfortunately, these two tertiary oximes were not pursued further, because quaternary pyridinium oximes (e.g., 2-PAM) were reported to be more potent reactivators of phosphonylated AChE by several orders of magnitude in human erythrocytes [see review by Hobbiger, 1963]. We recently showed that MINA reactivated AChE in the brain, reduced toxic signs, improved survival, and prevented or terminated seizures following GB intoxication in guinea pigs [Shih, et al., 2010a; Shih, et al., 2009a].

Two analogs of MINA were also selected for evaluation. N,N-diethyl-3-(2-(hydroxyimino)acetoxy)propan-1-aminium chloride (DHAP; ICD 4259) is an aliphatic tertiary oxime that was selected for synthesis and study because of its low toxicity, its relatively high reactivation efficiency of GB-inhibited AChE, and its having a PKa similar to that of 2-PAM [Benschop et al., 1976]. N-(2-(azepan-1-yl)ethyl)-2-(hydroxyimino)acetamide (RS194B) has been synthesized and investigated in the laboratories of Dr. Palmer Taylor and his associates (University of California at San Diego, La Jolla, CA). It was synthesized based on its enhanced intrinsic AChE reactivation potency, lower toxicity, and superior pharmacokinetic profile including faster CNS penetration and longer retention [Radic et al., 2012].

A pro-drug delivery concept had also been introduced earlier to overcome the limitation of quaternary 2-PAM. Bodor et al. [1976] were the first to synthesize a dihydropyridine derivative of 2-PAM, pro-2-PAM (6-((hydroxyimino)methyl)-1-methyl-2,5-dihydropyridinium chloride; dihydro-2-PAM) in an attempt to get an oxime across the BBB. Pro-2-PAM is oxidized to 2-PAM in the peripheral tissues and CNS. Subsequent studies in beagle dogs found that the conversion of pro-2-PAM to 2-PAM had a half-life ($t_{½}$) of 1.04 min and a biological $t_{½}$ of 168 min, 60 min longer than that of native 2-PAM [Shek et al., 1976a]. In a study using mice, ^{14}C-labeled pro-2-PAM was administered intravenously (i.v.), and the percentage of label was determined in the brain. It was found that 1.5% of the administered dose was detectable in the brain, and that pro-2-PAM was able to reactivate AChE inhibited by 0.5 x LD_{50} DFP (diisopropyl fluorophosphates; dyflos; fluostigmine) [Shek et al., 1976b]. Further studies evaluated pro-2-PAM for its efficacy against OP intoxication, though their results are ambiguous. Rump et al. [1978] showed that administration of pro-2-PAM significantly increased the convulsive dose of DFP by 1.83 times in mice. Clement [1979] found that while administration of pro-2-PAM resulted in higher levels of mouse brain AChE activity, there was no correlation with survival, and suggested that pro-2-PAM did not offer a significant improvement over 2-PAM in animals challenged with either DFP or the nerve agent GB. In another study, Talbot et al. [1986] found that pretreatment with pro-2-PAM provided better protection than 2-PAM against lethal GB intoxication in rats, but not against soman and VX. Gordon et al. in 2008 and Demar et al. in 2010 reported that pro-2-PAM protected brain AChE of guinea pigs challenged with DFP and also protected them from DFP-induced seizures, hypothermia and bradycardia. We recently showed that pro-2-PAM provided modest reactivation of GB- and VX-inhibited AChE in brain and periphery, which was also reflected by a minimum lethality protection and a limited ability to block or terminate seizures elicited by these agents, but was ineffective in reversing the effects of GF on AChE inhibition or seizures [Shih, et al., 2010c, 2011].

JK-3-38 (2-(hydroxyimino)-N,N-dimethyl-N'-butylacetimidamide hydrochloride) is one of the amidine-oxime reactivators recently synthesized in Dr.

John Cashman's laboratories (Human BioMolecular Research Institute, San Diego, CA). The concept used to develop this oxime relied on a combination of an amidine residue and oxime functionality whereby the amidine increases the binding affinity to the ChE and the oxime is responsible for reactivation. These compounds are reported to reactivate *in vitro* AChE inhibited by nerve agent surrogates, enter the CNS, and result in 100% 24-hour survival when given as pretreatment or administered 5 min after soman or GB surrogate exposure in mice [Kalisiak et al., 2011; 2012].

SWRI48A (2-(6-((1-benzylpiperidin-4-yl)methoxy)pyridine-3-yl)-2oxoacetaldehyde oxime hydrochloride) and SWRI53A (N-((1-benzylpiperidin-4-yl)methyl)2-(hydroxyimino)-3-oxobutanamide hydrochloride) are benzyl piperidine oximes. They were selected and synthesized by Southwest Research Institute (San Antonio, TX) based on the composite rank order of oximes on three *in vitro* criteria: 1) oxime reactivation of recombinant human AChE that had been inhibited by GF, 2) the dissociation constant for oxime inhibition of recombinant human AChE in the absence of nerve agent (i.e., how inhibitory the oxime is by itself), and 3) an estimate of oxime penetration into the CNS based on the Madin Darby Canine Kidney permeability cell model [Cho et al., 1989].

Diethyxime (S-[2-(diethylamino)ethyl]4-bromobenzo-thiohydroximate) is a non-quaternary oxime and was first reported by Kokshareva et al. [1977] to be a low-toxic universal antidote having potent AChE reactivation at central and peripheral sites against dimethyl dichlorovinyl phosphate (DDVP) intoxication in rats. They further showed that diethyxime readily penetrates the CNS; its level in the brain was twice as high as that in blood serum. It normalizes bioelectric activity (EEG) and functional state motoneurons of the spinal cord and prevents the development of deep disorders in ultrastructure of rats' spinal nerve fibers induced by OP compounds. Furthermore, this compound possesses a pronounced antidotal action in acute poisoning with OP and carbamate pesticides of different chemical structures, by restoring the function of the respiratory and cardiovascular systems [Kokshareva et al., 2005]. However, the *in vitro* and *in vivo* AChE reactivation measurements and the protective index studies with diethyxime in DFP-poisoned rats could not be observed [Kenley et al., 1981; Das Gupta et al., 1983; Dube et al., 1986]. The therapeutic efficacy of diethyxime in intoxication with OP nerve agents has not been reported.

The experiments presented here were designed to evaluate and compare the capability of these tertiary oximes to reactivate or restore AChE activity in discrete brain regions and peripheral tissues of guinea pigs, when administered at the time of maximal inhibition of this enzyme by the nerve agents [Shih et al., 2005]. The reactivation capability was tested initially with AChE activity inhibited by GB and followed, if successful, by AChE activity inhibited by GF or VX.

MATERIALS AND METHODS

Subjects

Male Hartley guinea pigs (Crl:(HA) BR COBS) weighing 250-300 g were purchased from Charles River Labs (Kingston, NY). They were housed in individual cages in temperature (21 ± 2°C) and humidity (50 ± 10%) controlled quarters that were maintained on a 12-hour light – dark schedule (with lights on at 0600 h). Laboratory chow and filtered tap water were freely available whenever the animals were in home cages.

Materials

Saline (U.S.P.), Attane™ (Isoflurane, U.S.P.), and heparin sodium were purchased from Braun Medical, Inc. (Irvine, CA), Minrad, Inc. (Bethlehem, PA), and U.S.P., Inc. (Rockville, MD), respectively. Pralidoxime chloride (2-PAM), USP (97 – 102%) was purchased from Spectrum Chemical MFG. Corp. (Gardena, CA). MINA (anti-pyruvic aldehyde 1-oxime, 98%), DAM (2,3-butanedione monoxime, ≥98%), acetylthiocholine iodide (≥98%), methylatropine nitrate, and atropine sulfate were purchased from Sigma-Aldrich (St. Louis, MO). Ellman's reagent (5,5'-dithio-bis-(2-nitrobenzoic acid); DTNB), bicinchoninic acid (BCA); and Protein Assay Reagent A and Reagent B were purchased from Pierce Biotechnology, Inc. (Rockford, IL). Pro-2-PAM, DHAP (>98%), diethyxime, MMB-4 dimethanesulfonate (methoxime; 1,1′-methylene-bis[4-(hydroxyimino) methyl] pyridinium dimethansulfonate), SWRI48A (>98%), and SWRI53A (>98%) were synthesized by Southwest Research Institute (San Antonio, TX). JK-3-38 was synthesized by Human BioMolecular Research Institute (San Diego, CA). RS194B was synthesized by Scripps Research Institute (La Jolla, CA). Sarin (GB; isopropyl methylphosphonofluoridate), cyclosarin (GF; cyclohexyl methylphosphonofluoridate), and VX (O-ethyl S-(2-(diisopropylamino)ethyl) methylphosphonothioate) were obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD). Nerve agents were diluted in saline prior to subcutaneous (s.c.) injection. Methylatropine nitrate and oxime compounds were prepared in saline individually for intramuscular (i.m.) injection. Injection volumes were 0.50 ml/kg for nerve agents and treatment drugs.

Reactivation Experiment

For purpose of comparing the AChE reactivation capability among tertiary oximes, we use only one toxic equivalent dose (1.0 x LD_{50}) of each nerve agent, due to their lethal nature in order to have enough survivors for the investigation. For the same reason, the duration of the interaction between oxime and the agent-inhibited enzyme was kept for a period of 45 min. The dose of 2-PAM (25 mg/kg, im) is equivalent to three autoinjector doses (as in Mark I Nerve Agent Antidote Kit) in a

70-kg person. The dose of MMB-4 was 26 mg/kg (58.0 µmol/kg, im), which was equivalent to the maximum three autoinjector doses to be given to a 70-kg person (based on HI-6 DiCl) [Clair et al., 2000]. All tertiary oximes were evaluated in a dose-dependent manner, based on their reported toxic doses and solubility in water. The 4 - 5 doses within each oxime were separated by a constant logarithmic interval, with the exception of JK-3-38.

One to 3 days prior to the experiment, control blood samples (~0.5 ml) were drawn using the toenail clip method [Vallejo-Freire, 1951] and collected into a 1.0-ml microfuge tube containing 50 µl of heparin sodium (15 U/ml) to determine baseline ChE activity in whole blood (WB) and red blood cells (RBC). On the day of the study, guinea pigs were pretreated with methylatropine nitrate (1.0 mg/kg, i.m.) 15 min prior to a nerve agent exposure to minimize peripheral toxic effects (i.e., clear the airway from mucus secretion). Methylatropine nitrate is a peripherally acting muscarinic receptor blocker that does not affect ChE activity. Animals were injected s.c. with either saline (0.5 ml/kg) or a 1.0 x LD_{50} dose of GB (42.0 µg/kg), GF (57.0 µg/kg), or VX (8.0 µg/kg). Fifteen min after nerve agent injection, when the inhibition of ChE activity by these nerve agents reached maximum [Shih et al., 2005], saline (0.5 ml/kg), 2-PAM (25.0 mg/kg), MMB-4 (26.0 mg/kg), MINA (22.1, 35.0, 55.5, 87.9, or 139.3 mg/kg), DAM (23.0, 41.0, 73.0, or 128.8 mg/kg), pro-2-PAM (12.5, 17.7, 25.0, or 35.3 mg/kg), DHAP (35.5, 50.0, 70.8, 100.0, or 141.3 mg/kg), JK-3-38 (5.6, 10.0, 18.0, 35.5, 50.0, 70.8, or 100.0 mg/kg), RS194B (10.0, 32.0, 56.0, or 100.0 mg/kg), SWRI48A (32.0, 56.0, 100.0, or 180.0 mg/kg), SWRI53A (32.0, 56.0, 100.0, or 180.0 mg/kg), or diethyxime (38.0, 60.3, 95.5, 151.4, or 240.0 mg/kg) was given i.m. Control animals received s.c. saline (no nerve agent) and i.m. saline (no oximes).

Sixty min after s.c. saline or nerve agent administration, the animals were deeply anesthetized with isoflurane and euthanized by decapitation. Blood (~0.5 ml) was collected into a 1.0 ml microfuge tube containing 50 µl of heparin sodium solution (15 U/ml). For the WB samples, 20 µl of blood was diluted 1:25 (v:v) in 1% Triton–X100 solution. For the RBC samples, the original blood sample was centrifuged for 5 min at 14,000 rpm, and 10 µl of the packed RBC was then diluted 1:50 in 1% Triton–X100 solution. Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord and striatum) and peripheral tissue (diaphragm, heart, and skeletal muscle) were dissected. Brain samples were diluted 1:20 (v:v), while peripheral tissue samples were diluted 1:5, in 1% Triton-X100 solution and then homogenized. The homogenates were then centrifuged (31,000 x g at 4^{o}C; 20 min for brain and 30 min for peripheral tissues), and the supernatant was decanted and kept frozen at -80^{o}C until AChE activity and protein concentration analyses according to the methods described elsewhere [Shih et al. 2005, 2009a].

Data analysis

AChE activity was initially expressed as μmol substrate hydrolysed/ml/min for RBC and WB and then converted to percentage of the individual animal's baseline AChE value that was obtained one to three days prior to the day of experiment. In peripheral tissues and brain regions the AChE activity was initially expressed as μmol substrate hydrolyzed/g protein/min and then expressed as percentage of the saline-treated control AChE value obtained on the day of experiment. The enzymatic activities of the treatment groups were then expressed as percentage of the saline/saline control group (mean ± SEM % of control value) within a nerve agent. Statistical analysis was performed using a one-way ANOVA to compare across tissues for basal AChE activity, among tissues across nerve agents, and across treatment groups for each nerve agent. A post-hoc Tukey test was used for multiple comparisons. Statistical significance is defined as $p < 0.05$. Only comparisons between each treatment group in which AChE activity was significantly higher than the activity in the nerve agent/saline-treated group are discussed. The significantly increased AChE activity (via reactivation) in oxime-treated groups was expressed as the % recovery of the saline/saline-treated control baseline activity above that of the remaining AChE activity in nerve agent-inhibited group (i.e., the % of control AChE activity in nerve agent-exposed and oxime-treated group minus the % of control AChE activity in nerve agent-exposed and saline-treated group).

We expressed the significant AChE reactivation data sets in two different ways after subjecting them initially to standard statistical procedure as described above to identify significant treatment groups. Firstly, we expressed the statistically significant ranges (from low dose to high dose) of percent of recovery of AChE activity by the nine oximes at the various doses following exposure to GB, GF, and VX in blood (RBC and WB), peripheral tissues (diaphragm, heart, and skeletal muscle), and brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord and striatum). The effects of two quaternary oximes 2-PAM and MMB-4 were also included for comparison. Table 1A, 1B, and 1C summarized this evaluation following exposure to GB, GF, and VX, respectively. A double dash in the cell on the table indicates the change was not significant. Secondly, we divided the AChE reactivation data into three tissue compartments: the brain, the peripheral tissues, and the blood components. A "+" sign was assigned for each of the brain regions, peripheral tissues, or blood components when an oxime treatment significantly reactivated nerve agent-inhibited AChE. For example, if an oxime treatment significantly reactivated AChE in all seven brain regions its cell received the maximum of seven "+" signs in all three peripheral tissues its cell received the maximum of three "+" signs, or in the two components of blood its cell received two "+" signs. When an oxime treatment did not significantly reactivate a nerve agent-inhibited AChE in any tissue, it was assigned a "-" sign. This evaluation is shown in Table 2.

RESULTS

General observations

MMB-4, 2-PAM, MINA, DAM, pro-2-PAM, DHAP, SWRI48A, and SWRI53A were investigated against all three nerve agents, GB, GF, and VX. RS194B, diethyxime, and JK-3-38 were studied only against GB, because we did not have a sufficient supply of RS194B to test against GF or VX; and after testing against GB, diethyxime showed minimum effectiveness in reactivating GB-inhibited brain AChE, while JK-3-38 was toxic and lethal in the presence of GB at doses of 50 mg/kg and above.

None of the animals exposed to 1.0 x LD_{50} of GB, GF, or VX and treated with saline (vehicle), MMB-4, 2-PAM, MINA, DAM, DHAP, SWRI48A, SWRI53A, or RS194B died prior to the time of tissue collection at sixty min after nerve agent intoxication. However, pro-2-PAM at 35.3 mg/kg induced severe toxic/lethal interactions with GB and GF, which caused 50% (3/6) and 58% (11/19) mortality with an average time to death after pro-2-PAM treatment of 32.0 $\pm$ 2.0 and 32.9 $\pm$ 3.0 min for GB- and GF-exposed group, respectively. Diethyxime at dose of 240 mg/kg and JK-3-38 at doses of 50 mg/kg and above also produced a severe toxic/lethal interaction with GB exposure. Following GB exposure, one out of 10 (10%) animals died 24 min after diethyxime treatment. JK-3-38 at 50.0, 70.8, 100.0, and 141.3 mg/kg caused 54% (6/11), 69% (9/13), 56% (5/9), and 100% (4/4) mortality, respectively, and the average time of death after JK-3-38 was 12.2 $\pm$2.1, 23.7 $\pm$ 3.4, 15.4 $\pm$1.4, and 7.8 $\pm$ 0.6 min, respectively. Even though pro-2-PAM, JK-3-38 and diethyxime produced toxicity at higher doses, the dose-dependent AChE reactivating capacity was preserved.

AChE reactivation

Evaluation by the percentage of significant recovery of AChE activity. The significant recovery of AChE activity (in % of saline/saline control) by the nine oximes at doses from low to high following exposure to GB, GF, and VX in blood (RBC and WB), peripheral tissues (diaphragm, heart, and skeletal muscle), and brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord and striatum) is shown in Table 1A, 1B, and 1C.

Following GB exposure (Table 1A), the significant percentage of AChE recovery in the blood and peripheral tissues for MMB-4 (26 mg/kg) treatment was 64.0 – 69.2 % and 40.8 – 51.7 %, respectively, and for 2-PAM (25 mg/kg) treatment was 53.1 – 58.3 % and 29.4 – 50.0 %, respectively. Both oximes are highly effective in reactivating peripheral AChE. No AChE reactivation in the CNS, however, was observed for these 2 quaternary oximes.

Among the tertiary oximes in the peripheral tissues, the percentage of AChE recovery for pro-2-PAM, JK-3-38, RS194B, and DHAP was comparable with that of 2-PAM or MMB-4. This reactivating capability was followed by that of diethyxime, MINA, DAM, and SWRI 48A. SWRI53A displayed the least AChE reactivating capability of all, showing 4.4% AChE recovery only in the heart tissue. The rank order of reactivating potency for blood displayed a very similar pattern as that for peripheral tissues, with the exceptions that diethyxime had a 14.7% AChE recovery only in the WB, and DAM showed no enzyme reactivation at all. In the CNS, the percentage of AChE recovery for MINA was two-fold that for DHAP, SWRI53A and SWRI48A, and was triple that for RS194B. The reactivating capacity of pro-2-PAM in the CNS was about two-thirds that of MINA, and diethyxime reactivated brain AChE only in the cortex. Thus, the overall rank order (from high to low) of the effectiveness in AChE reactivation in the CNS was MINA > pro-2-PAM > DHAP > SWRI48A = SWRI53A > RS194B = diethyxime, while DAM and JK-3-38 were completely ineffective. When comparing the reactivating potencies between peripheral and CNS compartments, pro-2-PAM, DHAP and RS194B displayed higher reactivating potency in the periphery than in the CNS, while MINA and SWRI48A showed comparable potency between these 2 compartments.

Following GF exposure (Table 1B), MMB-4 was able to reactivate AChE in the blood, peripheral tissues, and the spinal cord with 42.4 – 47.6 %, 15.2 – 30.6 %, and 19.8 % recovery of AChE activity, respectively. No AChE reactivation in the CNS or peripheral tissues was observed for 2-PAM. It showed significant AChE reactivation only in the WB with an 11.9% recovery of enzyme activity. Among the 6 tertiary oximes studied with GF, all except DAM were capable of reactivating AChE in the peripheral tissues. SWRI53A reactivated GF-inhibited AChE only in the heart, while SWRI48A reactivated AChE only in the skeletal muscle (24.9%). MINA showed the highest % recovery of AChE activity in the blood and peripheral tissues. DHAP caused moderate reactivation of AChE activity in the blood (3.8 – 8.2% recovery) and peripheral tissues (9.0 – 12.0% recovery). Only MINA and SWRI48A were capable of reactivating AChE in the CNS; they shared the same degree of reactivating effectiveness in the brain (8.1 – 17.6 % recovery of AChE activity).

Following VX exposure (Table 1C), the significant percentage of AChE recovery in the blood and peripheral tissue for MMB-4 treatment was 58.4 – 67.6 % and 13.2 – 34.6 %, respectively, and for 2-PAM treatment, 30.1 – 37.9 % and 17.8 – 22.3 %, respectively. Both oximes are highly effective in reactivating peripheral AChE. No AChE reactivation in the CNS, however, was observed for these 2 oximes. Among the 6 tertiary oximes studied with VX, only pro-2-PAM and MINA were able to reactivate AChE to the same degree in the blood, peripheral tissues, and brain regions, while DHAP reactivated AChE only in the blood and skeletal muscle with

less effectiveness. DAM, SWRI48A, and SWRI53A were not able to reactivate VX-inhibited AChE in any peripheral and CNS tissues.

Evaluation by the numbers of tissues showing significant recovery of AChE activity. Table 2 summarizes the statistically significant increases in AChE activity resulting from oxime treatment at various doses following exposure to GB, GF, and VX in terms of the number of tissues in the brain regions, the peripheral tissues, and the blood components. Pro-2-PAM at 25 mg/kg was able to reactivate AChE in one brain region (cortex) following GB exposure and 3 brain regions (cortex, hippocampus, and spinal cord) following VX exposure. It was, however, not effective in reactivating GF-inhibited AChE in any brain region. In the peripheral tissues and blood, pro-2-PAM was equally effective in reactivation of both GB- and VX-inhibited AChE in all 3 tissues and blood components, starting at a dose of 12.5 mg/kg. Pro-2-PAM reactivated GF-inhibited AChE only in the heart and WB. The relative AChE reactivating potency of pro-2-PAM against these nerve agents was GB = VX > GF. As mentioned earlier pro-2-PAM at 35.3 mg/kg produced severe toxic/lethal interactions with GB and GF. This dose, however, didn't dampen its AChE reactivating ability in survivors, as more CNS tissues displaying significant AChE recovery.

It is striking to notice that MINA was capable of reactivating AChE inhibited by all three nerve agents in at least 5 brain regions. Following GB exposure, doses of MINA at 55.5 mg/kg and above were able to significantly reactivate inhibited brain AChE. As the dose of MINA increased the AChE activity was reactivated in more brain regions. At the highest dose of 139.3 mg/kg, all seven brain regions displayed significant AChE reactivation. MINA also began to show its ability to reactivate AChE when the dose reached 55.5 mg/kg and above following GF exposure, and at 139.3 mg/kg a total of six regions, all except the striatum, showed significant AChE reactivation. In the case of VX, MINA, at doses of 55.5 and 87.9 mg/kg, significantly reactivated AChE only in the hippocampus. When the dose reached 139.3 mg/kg, five brain regions, with the exception of the striatum and spinal cord, displayed significant AChE reactivation. The relative brain AChE reactivating potency of MINA against these nerve agents was GB > GF = VX. In the peripheral tissues MINA reactivated AChE inhibited by GB and VX in all three tissues at 139.3 mg/kg, and AChE inhibited by GF only in 2 tissues (diaphragm and heart). In the blood components (RBC and WB), MINA reactivated GB- and VX-inhibited AChE in both components at 139.3 mg/kg, while at 35.0 mg/kg and above, MINA reactivated GF-inhibited AChE only in the WB. The capability of MINA to reactivate AChE activity in peripheral tissues and blood inhibited by these three nerve agents was in the order of GB > VX > GF.

DAM was not effective in reactivating brain AChE following exposure to GB, GF, or VX. It reactivated only GB-inhibited AChE in the skeletal muscle at 23.0, 41.0, and 128.8 mg/kg and GF-inhibited AChE in the WB at 128.8 mg/kg.

DHAP reactivated GB-inhibited AChE in 5 brain regions, with the exception of the striatum and spinal cord, at 100 mg/kg. It was also very effective in reactivating AChE in the peripheral tissues and blood at all doses following exposure to GB. Following exposure to GF, DHAP was not able to reactivate AChE in any brain regions, and showed reactivation only in the skeletal muscle and WB at doses 100.0 mg/kg or higher. It was, however, effective in reactivating AChE inhibited by VX in both blood components at 100 mg/kg, but not effective in reactivating AChE in any peripheral or CNS tissues. Therefore, DHAP was predominately effective only in reactivation of GB-inhibited AChE.

Both SWRI48A and SWRI53A reactivated GB-inhibited AChE in 6 and 7 brain regions, respectively, at higher doses (100.0 and 180.0 mg/kg). SWRI48A, like MINA, was capable of reactivating GF-inhibited AChE in the CNS (3 brain regions: cortex, midbrain, and striatum); SWRI48A at all doses tested also reactivated blood AChE inhibited by GF. SWRI53A at higher doses reactivated GF-inhibited AChE only in the heart and WB. Neither oxime was capable of reactivating VX-inhibited AChE in any peripheral or CNS tissues. Thus, SWRI48A was a better AChE reactivator than SWRI53A in the peripheral tissues and blood.

Following GB exposure, JK-3-38 showed no capability to reactivate CNS AChE. It reactivated GB-inhibited AChE in all 3 peripheral tissues at 35.5 mg/kg and above and 2 blood components at alldoses tested. JK-3-38 at 50.0 mg/kg and above produced severe toxic/lethal interactions with GB exposure. RS194B at 32.0 mg/kg and above reactivated AChE in all 3 peripheral tissues and 2 blood components, but it required 100.0 mg/kg to reactivate AChE in the 6 brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain and striatum). Diethyxime was relatively ineffective in reactivating AChE in the CNS; it reactivated AChE activity only in the cortex at 151.4 mg/kg. However, it reactivated AChE activity in all 3 peripheral tissues at 151.4 mg/kg and above.

DISCUSSION

The toxic and lethal consequences of OP nerve agent intoxication are due to irreversible inhibition of the critical cholinergic enzyme AChE that serves to hydrolyze and degrade the released cholinergic neurotransmitter ACh [Taylor, 2001]. The currently used antidotes include an oxime reactivator (such as 2-PAM, obidoxime, or HI-6) to rescue the activity of nerve agent-inhibited AChE. However, these oximes have quaternary nitrogen structures and do not readily penetrate the BBB to reactivate brain AChE, limiting their therapeutic action to only peripheral

AChE [Shih et al., 2009b]. Thus, the nerve agent-induced central neurobehavioral adverse effects have to be mitigated in general by supplemental administration of an anticholinergic such as atropine sulfate and a benzodiazepine such as diazepam or midazolam [Wilson and Ginsburg, 1955; Moore et al., 1995; Taylor, 2001; Aas 2003]. These drugs themselves also cause psycho-pharmacological and sedative effects. It is our belief that the best nerve agent antidote is an AChE reactivator that can restore normal cholinergic neurotransmission both in the periphery and in the CNS after nerve agent exposure.

In this study, we purposely did not conduct experimental exposures with the OP nerve agents tabun and soman for the following reasons. Oxime moieties can restore inactivated AChE by nucleophilic displacement of the OP moiety from the active-site serine, provided that they can access the phosphoserine linkage. The chemical structure of tabun with its amide nitrogen makes this particularly problematic. The relative inability of oximes to restore tabun-inhibited AChE has been attributed, at least in part, to nuclophilic impedance [Ekstrom et al., 2006a; b; Hoskovcova et al., 2007]. Other AChE-inhibitors, such as soman, become refractory to reactivation by an 'aging' process [Fleisher and Harris, 1965; Fleisher et al., 1967]. At some point after inactivation, nerve agents can undergo dealkylation, resulting in the formation of negatively charged methylphosphonate AChE, which is held to present an electrostatic barrier to nucleophilic attack by oximes. This, combined with structural shifts in this OP-AChE complex, renders the AChE-OP complex irreversible. Soman aging is extremely rapid, with a half life of 1–8 min depending on species [Talbot et al., 1988; Luo et al., 2007]. At the time of oxime administration in this experiment, the OP-AChE complex would likely be irreversible in an animal exposed to soman. In contrast, the half-life of reactivatable OP-AChE exceeds 3 h for GB and is even longer for GF and VX [Worek et al., 2004; Luo et al., 2007].

MINA, a tertiary oxime, has been shown to reactivate AChE within the CNS, reduce toxic signs, improve survival, and prevent or spontaneously terminate seizures following GB intoxication [Rutland, 1958; Askew 1956a, 1957; Dultz et al., 1957; Myers, 1959; Wills, 1959; Shih et al., 2009a, 2010a]. We recently confirmed and extended those earlier reports of the beneficial effects of MINA against GB reported earlier [Skovira et al., 2010] to GF and VX. Our results demonstrate the benefit of reactivating brain AChE with a CNS-active oxime, such as MINA, on survival outcomes following nerve agent intoxication [Skovira, et al. 2010]. It was observed that the more AChE activity present in the brain following MINA treatment, the greater the oxime's survival efficacy against nerve agent intoxication. Limited reactivation of CNS AChE inhibited by a nerve agent corresponded to little or no increase in the protective efficacy of increasing doses of MINA. As the amount of CNS AChE reactivation in multiple brain regions increased against GF with higher doses of MINA, so too did MINA's protective efficacy. Extensive reactivation of CNS AChE by higher doses of MINA was strongly related to a

marked increase in the protective ratio of increased doses of this oxime against GB [Skovira et al., 2010]. However, the percentage of AChE activity being reactivated in the peripheral tissues by MINA was relatively small when compared with that reactivated by 2-PAM or other quaternary oximes on the equi-molar basis. Much higher doses for MINA are required to achieve the same degree of AChE reactivation in the periphery as achieved with 2-PAM. Askew [1956b] reported that the toxicity of MINA was mostly due to the generation of hydrogen cyanide, limiting its therapeutic application. Therefore, we conducted the present study to further evaluate and compare the capability of MINA and eight other tertiary oximes to reactivate CNS AChE when given after the maximal inhibition of this enzyme by the nerve agents GB, GF, and VX was reached [Shih et al., 2005]. The goal is to identify a safe CNS penetrating oxime with a broad-spectrum reactivating capacity for all OP nerve agents.

Under the conditions of this study, both quaternary (2-PAM, MMB-4) and all 9 tertiary oximes were able to reactivate GB-, GF-, or VX-inhibited AChE, but with notable variations with respect to the 3 nerve agents. In the periphery, the quaternary oxime MMB-4 was capable of reactivating AChE inhibited by all three nerve agents in the blood and peripheral tissues. 2-PAM, on the other hand, reactivated GB- and VX-inhibited AChE in the blood and peripheral tissues, but only reactivated GF-inhibited AChE in WB. As expected, similar to 2-PAM, the tertiary oxime pro-2-PAM reactivated GB- and VX-inhibited AChE in the blood and peripheral tissues with the same potency, however, pro-2-PAM was also able to reactivate GF-inhibited AChE in the heart, in addition to WB. DHAP was an excellent reactivator of GB-inhibited AChE in the blood and peripheral tissues, but was a very weak reactivator of GF- and VX-inhibited AChE. Both SWRI48A and SWRI53A induced only moderate action in reactivating GB- and GF-inhibited AChE in the blood and peripheral tissues and had no effect on VX-inhibited AChE. Although DAM produced a moderate effect in reactivating GB-inhibited AChE in the periphery, it was totally ineffective on AChE inhibited by GF and VX. JK-3-38 and RS194B were excellent reactivators, while diethyxime was a moderate reactivator of GB-inhibited AChE. Among the 9 tertiary oximes compared, MINA was the only reactivator consistently producing moderate AChE recovery in the blood and peripheral tissues following GB, GF and VX intoxication. In the CNS, MINA was similarly the most effective reactivator among the 9 tertiary oximes, increasing AChE activity across multiple doses and in multiple brain regions against all three nerve agents. Pro-2-PAM, DHAP, SWRI-53A SWRI-48A, and RS194B were able to reactivate GB-inhibited brain AChE, but had different specificity for either GF- or VX-inhibited AChE in the CNS. SWRI48A was also capable of reactivating GF-inhibited brain AChE, but not VX-inhibited AChE. On the other hand, pro-2-PAM reactivated VX-inhibited AChE in the brain, but not GF-inhibited AChE. Diethyxime induced a mild reactivation (6.9% recovery) of AChE inhibited by GB

only in the cortical region at a relatively high dose (151.4 mg/kg). Its excellent penetration into the CNS and reactivation of OP nerve agent-inhibited brain AChE reported earlier [Kokshareva et al., 1977, 2005] could not be confirmed by our data. DAM did not reactivate brain AChE inhibited by any of the 3 nerve agents.

Our results showed that pro-2-PAM, JK-3-38, and diethyxime had toxic/lethal interactions with the nerve agent GB and GF at doses that generate the most optimal recovery of peripheral AChE activity, indicating a low margin of safety as an antidote for nerve agent intoxication. The reasons for the lethal interaction between these oximes and nerve agent are unknown. Although DHAP, SWRI48A, SWRI53A were good reactivators of AChE inhibited by GB, they were not effective against either GF- or VX-inhibited AChE. DAM was completely ineffective in reactivating brain AChE inhibited by all 3 nerve agents when given 15 min after nerve agent exposure. This is in contrast to what we had observed when it was administered 5 min after the same challenge dose of GB [Shih et al., 2009a]. The reason for this is not clear. RS194B was an excellent reactivator of GB-inhibited AChE, achieving the same degree of AChE recovery in peripheral tissues as did 2-PAM. Its capacity to reactivate GB-inhibited AChE in the CNS was only a third that of MINA. The ability of RS194B to reactivate GF- or VX-inhibited AChE is awaiting investigation. Thus, at this moment, MINA remains the most broad spectrum centrally active AChE reactivator we have evaluated.

MINA showed a dose-dependent reactivation of AChE activity in both central and peripheral tissues against 1.0 x LD_{50} challenge dose of each nerve agent. As the dose of MINA increased, more inhibited AChE was reactivated in more tissues. MINA was, however, a weaker reactivator of VX- and GF-inhibited AChE than of GB-inhibited AChE, and a weaker reactivator of peripheral AChE than were the quaternary oximes (such as 2-PAM or MMB-4). As reported, tertiary oximes like MINA are much less potent reactivators of AChE than are mono-pyridinium and bis-pyridinium oximes [Askew, 1956a; Hobbiger, 1963]. Therefore, it was not surprising that the effective reactivating doses of MINA used in this study were 3-12 times higher on a molar basis than the dose of 2-PAM (145.0 μmol/kg). Metabolism of MINA generates cyanide, which is thought to be responsible for the toxic effect of this oxime [Askew, 1956b]. However, the highest dose of MINA (139.3 mg/kg) used in the present study was not a lethal dose and no toxic drug interactions were observed in guinea pigs.

Since the toxic effects of VX take a longer time to develop [Shih et al., 2003, 2007], an oxime reactivator would be expected to have the advantage of reaching the synaptic and neuromuscular junctional AChE pools, placing it in prime position at the time AChE inhibition by VX begins. However, this was not the case in the present study. The AChE reactivation capacity in the presence of VX in the CNS was only observed in very limited brain regions and only at very high doses of MINA when compared with that in the presence of GB. The reason for the weaker

action of MINA in reactivating VX-induced AChE activity was unclear, but could be due to several factors. The most likely explanation is that MINA has less affinity and reactivity as a reactivator of VX-inhibited AChE than it has as a reactivator of GB-inhibited AChE. Pharmacodynamic factors could also play a role. Oximes, in general, have relatively short half-lives in the body [Green et al., 1986]. It is possible that the concentration and residence time of MINA at the synaptic and neuromuscular junctions are not sufficient to reactivate VX-inhibited AChE. This would explain why much higher doses were needed.

It is unclear why MINA and DHAP reactivated GF-inhibited AChE in these studies. Reactivation by oximes of GF-inhibited AChE, in general, is more difficult than that of GB- and VX-inhibited AChE [Shih et al., 2009b]. Some very potent oximes (relative to MINA and DHAP), such as 2-PAM and obidoxime are poor reactivators of GF-inhibited AChE [Shih et al., 2009b]. DHAP, a structure analog of MINA, reactivated GF-inhibited AChE in the blood and peripheral tissues similar to MINA, but with weaker reactivating capacity than MINA (12.0 % vs. 34.7 % AChE recovery). SWRI48A and SWRI53A were selected based on the rank order of *in vitro* oxime reactivation of recombinant human AChE that had been inhibited by GF. Both of them reactivated GF-inhibited AChE in blood and selected peripheral tissues; however, only SWRI48A reactivated AChE in the CNS.

In conclusion, AChE reactivators, such as oximes, play a critical role in the treatment of OP nerve agent intoxication. Arguably, they are the most important component of nerve agent treatment regimens because they can restore the activity of AChE. The other therapeutic components (atropine sulfate and anticonvulsant drug) provide supportive benefit and facilitate the effectiveness of the oxime. Our earlier results demonstrated that protective effectiveness is significantly enhanced if nerve agent-inhibited AChE in the CNS is also reactivated [Skovira et al., 2010]. The benefits of a centrally acting oxime may not be limited to improving survival; these oximes may also be important in preventing and/or terminating seizures and preventing seizure-related neuropathology [Shih et al., 2009a, b, 2010a, b]. Like the quaternary oximes that exhibit different reactivation capacities against AChE inhibited in the periphery by different nerve agents, tertiary oximes also show different reactivation capacities against AChE inhibited by specific nerve agent in the periphery or in the CNS. Since beginning our search in the late 1990s for an effective CNS active oxime, we have found MINA to be among the best of the 9 tertiary oximes we have evaluated. It showed the broadest spectrum of action among these tertiary oximes in reactivating AChE inhibited by GB, GF and VX, although the degree of reactivation against GF and VX was much weaker than against GB. Despite being the best of the tertiary oximes, MINA displayed weaker AChE reactivating capacity in the peripheral tissues than did the quaternary oximes. The potential that it generates toxic cyanide gas may limit its usefulness, although in our study its intrinsic toxic signs and its toxic interaction with nerve agents were not

observed. Because of these deficiencies associated with MINA, further research and development should continue to identify a safer AChE reactivator that can readily penetrate the CNS and provide broad spectrum benefits for all nerve agents.

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Figure 1.
Chemical Structures of Tertiary Oximes

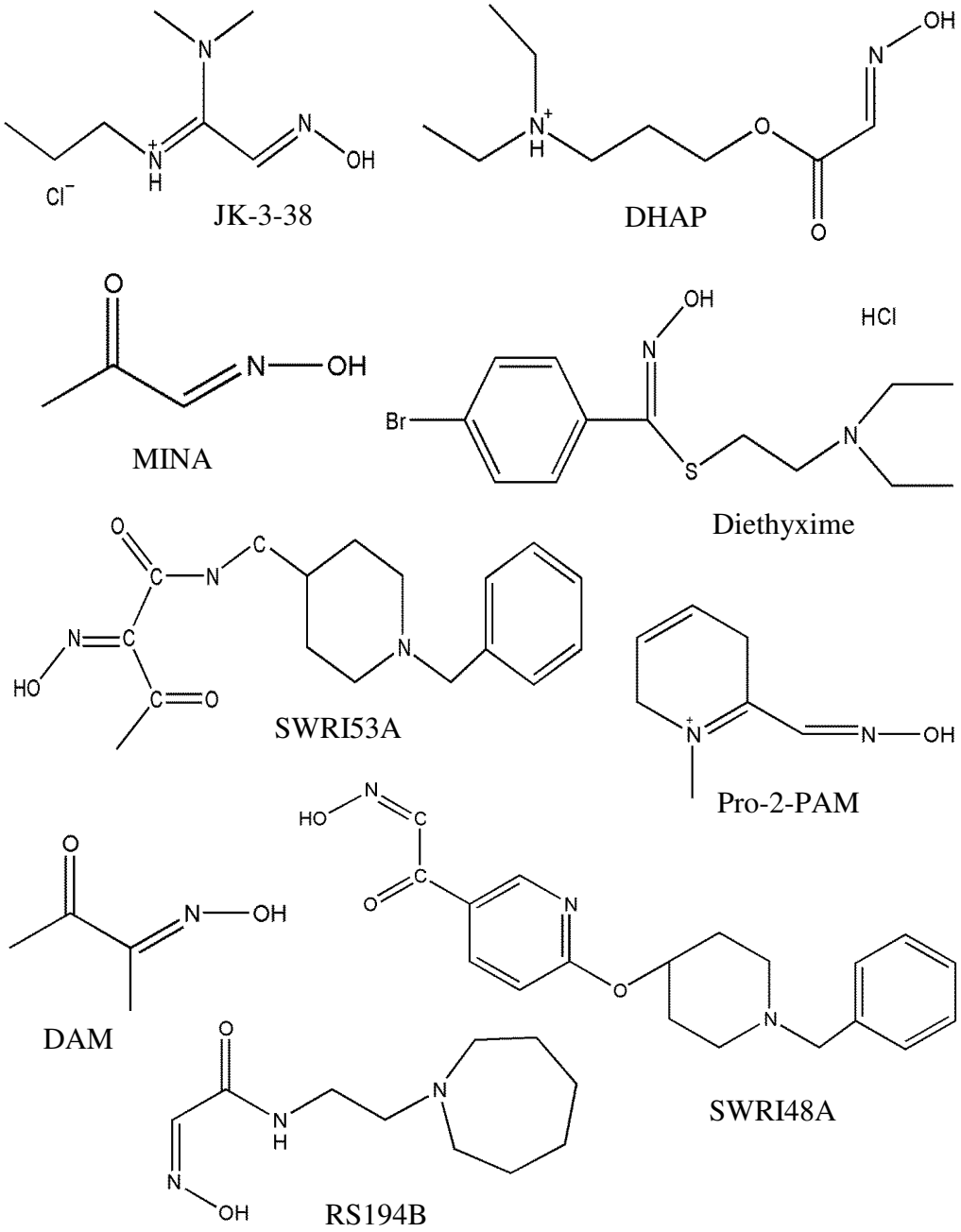

Table 1. Significant percentage of control AChE activity recovery in blood, peripheral tissue, and brain regions by oxime treatments at various doses following exposure to sarin, cyclosarin, and VX[1]

1A. Following sarin exposure

Sarin (GB)				
Oxime	**Dose Range (mg/kg)**	**Significant AChE % Recovery**		
		Blood	Peripheral Tissues	Brain Regions
MMB-4	26.0	64.0 - 69.2	40.8 - 51.7	--
2-PAM	25.0	53.1 - 58.3	29.4 - 50.0	--
Pro-2-PAM	12.5 - 35.3	17.9 - 36.1	21.2 - 68.0	9.7 - 19.2
MINA	22.1 - 139.3	11.1 - 34.4	10.0 - 24.1	14.5 - 31.2
DAM	23.0 - 128.8	--	17.6 - 19.1[c]	--
DHAP	35.5 - 141.3	10.9 - 32.0	14.6 - 46.8	6.1 - 17.9
SWRI48A	32.0 - 180.0	3.4 - 5.5	3.5 - 14.1	4.6 - 13.5
SWRI53A	32.0 - 180.0	1.7 - 3.6	4.4[b]	5.4 - 13.4
JK-3-38	5.6 - 100.0	5.2 - 45.2	21.0 - 59.7	--
RS194B	10.0 - 100.0	16.1 - 46.3	23.1 - 57.5	2.9 - 10.1
Diethyxime	38.0 - 240.0	14.7[a]	10.5 - 24.1	6.9[d]

1B. Following cyclosarin exposure

Cyclosarin (GF)				
Oxime	**Dose Range (mg/kg)**	**Significant AChE % Recovery**		
		Blood	Peripheral Tissues	Brain Regions
MMB-4	26.0	42.4 - 47.6	15.2 - 30.6	19.8[e]
2-PAM	25.0	11.9[a]	--	--
Pro-2-PAM	12.5 - 35.3	6.9 - 13.1	9.2 - 18.5	--
MINA	22.1 - 139.3	13.8 - 37.3	11.7 - 34.7	8.1 - 16.7
DAM	23.0 - 128.8	9.6[a]	--	--
DHAP	35.5 - 141.3	3.8 - 8.2	9.0 - 12.0	--
SWRI48A	32.0 - 180.0	3.1 - 5.4	24.9[c]	10.2 - 17.6
SWRI53A	32.0 - 180.0	4.2 - 6.4	10.7[b]	--

1C. Following VX exposure

VX				
	Dose Range	**Significant AChE % Recovery**		
Oxime	**(mg/kg)**	Blood	Peripheral Tissues	Brain Regions
MMB-4	26.0	58.4 - 67.6	13.2 - 34.6	--
2-PAM	25.0	30.0 - 37.9	17.8 - 22.3	--
Pro-2-PAM	12.5 - 35.3	13.7 - 28.0	11.5 - 38.8	8.2 - 18.3
MINA	22.1 - 139.3	15.0 - 33.9	8.1 - 21.8	10.4 - 23.2
DAM	23.0 - 128.8	--	--	--
DHAP	35.5 - 141.3	8.4 - 10.9	15.4[c]	--
SWRI48A	32.0 - 180.0	--	--	--
SWRI53A	32.0 - 180.0	--	--	--

[1] Guinea pigs were pretreated with methylatropine nitrate (2 mg/kg, i.m.) 15 min prior to challenge with a subcutaneous dose (1.0 x LD_{50}) of a nerve agent, followed 15 min later with an intramuscular dose of the oxime. Tissues were collected 60 min after nerve agent challenge.

Data in each cell show the statistically significant % of AChE recovery in three tissue components (blood, peripheral tissues, and brain regions) following oxime treatment at various doses from low to high doses tested.

[a,b,c,d,e] indicate significant AChE reactivation in a single tissue: [a]WB; [b]heart; [c]skeletal muscle; [d]cortex; and [e]spinal cord.

-- (double dash line) indicated there was no significant AChE reactivation in the tissue component by the range of doses of oxime tested

Table 2. Number of tissues in brain, periphery and blood showing significantly increased AChE activity after oxime treatment at various doses following exposure to saline (GB), cyclosarin (GF), and VX[1,2]

	Pro-2-PAM (12.5)	Pro-2-PAM (17.7)	Pro-2-PAM (25.0)	Pro-2-PAM (35.3)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)				
GB	-	+	+	++
GF	-	-	-	-
VX	-	+	+++	++++
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)				
GB	+++	+++	+++	+++
GF	+	+	+	+
VX	++	++	+++	+++
(C) Blood (red blood cells and whole blood)				
GB	++	++	++	++
GF	+	+	+	+
VX	+	++	+	++

	MINA (22.1)	MINA (35.0)	MINA (55.5)	MINA (87.9)	MINA (139.3)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)					
GB	-	-	+++++	++++++	+++++++
GF	+	-	++++	+++	++++++
VX	-	-	+	+	+++++
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)					
GB	-	-	++	++	+++
GF	-	+	++	++	++
VX	+	-	+	++	+++
(C) Blood (red blood cells and whole blood)					
GB	-	-	++	++	++
GF	-	+	+	+	+
VX	-	-	-	-	++

	DAM (23.0)	DAM (41.0)	DAM (73.0)	DAM (128.8)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)				
GB	-	-	-	-
GF	-	-	-	-
VX	-	-	-	-
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)				
GB	+	+	-	+
GF	-	-	-	-
VX	-	-	-	-
(C) Blood (red blood cells and whole blood)				
GB	-	-	-	-
GF	-	-	-	+
VX	-	-	-	-

	DHAP (35.5)	DHAP (50.0)	DHAP (70.8)	DHAP (100.0)	DHAP (141.3)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)					
GB	+	+	+++	+++++	+++
GF	-	-	-	-	-
VX	-	-	-	-	-
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)					
GB	++	++	+++	+++	+++
GF	-	-	-	+	+
VX	-	+	-	-	-
(C) Blood (red blood cells and whole blood)					
GB	++	++	++	++	++
GF	-	-	+	+	+
VX	-	-	-	++	+

	SWRI-53A (32.0)	SWRI-53A (56.0)*	SWRI-53A (100.0)	SWRI-53A (180.0)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)				
GB	-	-	+++++++	+++++++
GF	-	-	-	-
VX	-	-	-	-
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)				
GB	-	-	-	+
GF	-	-	-	+
VX	-	-	-	-
(C) Blood (red blood cells and whole blood)				
GB	+	-	+	+
GF	-	-	+	+
VX	-	-	-	-

	SWRI-48A (32.0)	SWRI-48A (56.0)	SWRI-48A (100.0)	SWRI-48A (180.0)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)				
GB	++	++	+++++	++++++
GF	-	-	-	+++
VX	-	-	-	-
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)				
GB	+	+	++	++
GF	-	+	-	-
VX	-	-	-	-
(C) Blood (red blood cells and whole blood)				
GB	-	-	++	++
GF	++	++	++	+
VX	-	-	-	-

	JK-3-38 (5.6)	JK-3-38 (10.0)	JK-3-38 (18.0)	JK-3-38 (35.5)	JK-3-38 (50.0)	JK-3-38 (70.8)	JK-3-38 (100.0)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)							
GB	-	-	-	-	-	-	-
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)							
GB	+	++	++	+++	+++	+++	+++
(C) Blood (red blood cells and whole blood)							
GB	++	++	++	++	++	++	++

	RS194B (10.0)	RS194B (32.0)	RS194B (56.0)	RS194B (100.0)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)				
GB	-	+	+	++++++
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)				
GB	-	+++	+++	+++
(C) Blood (red blood cells and whole blood)				
GB	-	++	++	++

	Diethyxime (38.0)	Diethyxime (60.3)	Diethyxime (95.5)	Diethyxime (151.4)	Diethyxime (240.0)
(A) Brain regions (brainstem, cerebellum, cortex, hippocampus, midbrain, spinal cord, striatum)					
GB	-	-	-	+	-
(B) Peripheral tissues (diaphragm, heart, skeletal muscle)					
GB	+	+	+	+++	+++
(C) Blood (red blood cells and whole blood)					
GB	-	-	-	+	-

[1] Guinea pigs were pretreated with methylatropine nitrate (2 mg/kg, i.m.) 15 min prior to challenge with a subcutaneous dose (1.0 x LD_{50}) of a nerve agent, followed 15 min later with an intramuscular dose of the oxime. Tissues were collected 60 min after nerve agent challenge.

[2] The significant AChE reactivation data were divided into three tissue compartments: the brain regions, the peripheral tissues, and the blood components. A "+" sign was assigned for each of the brain regions, peripheral tissues, or blood components when an oxime treatment significantly reactivated nerve agent-inhibited AChE. For example, if an oxime treatment significantly reactivated AChE in all seven brain regions its cell received the maximum of seven "+" signs in all three peripheral tissues its cell received the maximum of three "+" signs, or in the two components of blood its cell received two "+" signs. When an oxime treatment did not significantly reactivate a nerve agent-inhibited AChE in any tissue, it was assigned a "-" sign.

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